IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Ebrahim ZANDI, et al.

Title: COMPOSITION AND METHOD FOR RECONSTITUTING IkB KINASE IN YEAST AND METHODS OF USING SAME

Appl. No.: 10/079,949

Filing Date: 2/19/2002

Examiner: Prouty, Rebecca E.

Art Unit: 1652

Confirmation Number: 6542

DECLARATION UNDER 37 CFR SECTION 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. We, Ebrahim Zandi and Beth Schomer Miller, hereby declare as follows.

2. We are the Ebrahim Zandi and Beth Schomer Miller, who are named as co-inventors of the above-identified application.

3. That we conceived and reduced to practice in the United States the transformation of an IKK subunit gamma (γ) gene, an IKK subunit alpha (α) gene and/or an IKK subunit beta (β) gene into yeast and the separation from that yeast a substantially homogenous and biologically functional IKK protein complex prior to November 15, 2000, the online publication date of the literature article Li et al. (2001) "Role of IKKγ/NEMO in Assembly of the IκB Kinase Complex"

Journal of Biological Chemistry 276(6):4494-4500. Attached hereto is Exhibit A, a copy of pages from laboratory notebooks recorded by Beth Schomer Miller working under our direct control and supervision showing a reduction to practice wherein the activity of a purified IKK complex from yeast transformed with either IKKβ, IKKβγ, or IKKαβγ compared to mammalian IKK complex isolated from control Hela cells or TNF stimulated HELA cells was determined. These experimental results demonstrate that a yeast cell was transformed with an IKK subunit gamma (γ) gene, an IKK subunit alpha (α) gene and/or an IKK subunit beta (β) gene. The yeast was then grown and a substantially homogenous and biologically functional IKK protein complex was separated from the yeast.

4. That the documents in Exhibit A, which relates to the aforementioned actual reduction to practice, are exact and true copies. All personal information, including names and dates have been redacted from the documents, but all dates are prior to November 15, 2000.

5. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are true; and further that all statements made herein are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United Sates Code, and that such false statements may jeopardize the validity of the patent application currently being examined and any patent issued thereon.

Respectfully submitted,

Ebrahim Zandi

Ebrahim Zandi — 06/24/2008

Signature: Date:

Beth Schomer Miller

Signature: Date:

Atty. Dkt. No. 064189-0501

Beth Schomer Miller

Beth Schomer Miller
Signature:

June 25, 2008
Date:

Exhibit A

TOPS 36161
MADE IN U. S. A.

[redacted] Purpose: to compare IKK activity in
y αβγ yβγ yβ HNS TNF

Butw GF βγ 8-25 good (HA) signal in 2 sec exp.
5 µL Fr 10 or 11

Butn GF αβγ good α + γ 1 min 5 µL Fr 10-11
1 µ y αβγ
HA good signal 15 sec similar to

1 µ of [redacted] γ→βγ Fr 10

Butn β HA detect some Fr 15 after 1 min (15 µ)

HNS GF [redacted]
HNS β detected in 10 + 11 aft 1 min 20 µ
TNF weakly detected in 10 + 11 40 min 20 µ
INP. identical.

Hugo's westerns were also poor for detection of
β in β alone + αβγ in his assays.
I'll have to play around with amounts

HNS Q20 + TNF Q20 were separated by gel filtration
INP. could detect ~~[illegible]~~ 5-15 µ by α IKK β + γ western
in 15 sec.

Less present than 5 µ y IKK

Put fractions in gel filtration → ~10 fold dilution
would need to use 150 µ for same amt.
Concentrate 150(10) + 150(11)
~~300~~ µ → 30 µ

use: 5, 10, 15

TOPS 36161
MADE IN U. S. A.

[redacted]

β-HA fraction 15 G.F.
3λ + 12λ 1x KA
5λ + 10λ 1x
10λ + 5λ 1x

βγ -HA Fr 10
3 + 12λ 1x
5 + 10λ 1x
10 + 5λ 1x

αβγ Fr 10-11
3 + 12λ 1x
5 + 10λ 1x

Load 35λ each
1 empty
2 empty
3 β3
4 5
5 10
6 βγ 3
7 5
8 10
9 αβγ 3
10 5
11 HNS 5
12 10
13 15
14 TNF 5
15 10
16 15
17 mw

all correctly loaded

HNS Q 20 → Sp 6 GF 10+11
200 + 200 → 40λ
use 5, 10, 15λ
+ 10λ + 5λ 1x

ULTAFREE MC
30. KD
put
300λ 1x kinase buffer in bottom
↓
prevent drying
o/n

TNF Q 20 → Sp 6 GF 10+11
200λ + 200λ → 40λ
use 5, 10, 15λ
+ 10λ + 5λ 1x

Should recover
≤ 40λ retentate
recover
25λ HNS add 15λ 1x
40λ TNF

1. aliquot extract + buffer according to table
2. Add 30λ kinase cocktail Inc 30' 30°C
3. Add ~~9λ~~ 6x SDS PAGE, heat — only 6λ added
4. Load 10% gel

Cocktail - 15
10x Kinase 45
20mM DTT 45
200μM ATP 45
0.5mg/ml GST-[illegible] 30λ
γ ATP 7.5λ 906-58
H2O 277.5

3λ 906-5[illegible]
4.5λ 58

[redacted] Purpose: to compare acidity of yB vs yBr vs yaBr vs HNS vs TNF

10% gel (10.19)			Stock
30% acryl		5 ml	1.05
BB		3.75	1.9 (6.8)
H_2O		6.25	4.5
APS		200 µl	75
TEMED		20 µl	10

File/Range: D:\Users\1012bsm.gel / 0.000-45853 Counts / 1.000000
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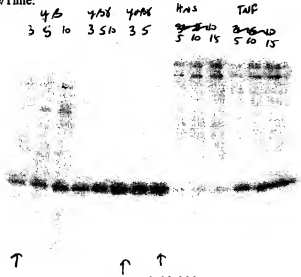
Image Name: D:\Users\1012bsm.gel
Image Comment: yeast b bg abg HNS TNF-Hela
scanned 9:13 am to2:05 pm

Present Date/Time [redacted]
Scan Date/Time:
Prep. Date/Time:



range 1-10,000

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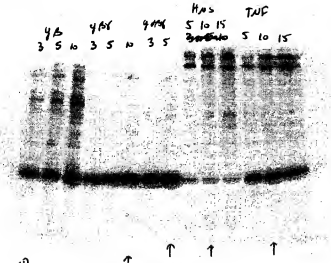
User Name: phospho
Image Name: D:\Users\1012bsm.gel
Image Comment: yeast b bg abg HNS TNF-Hela
scanned 9:13 am to2:05 pm

Present Date/Time: [redacted]
Scan Date/Time:
Prep. Date/Time:



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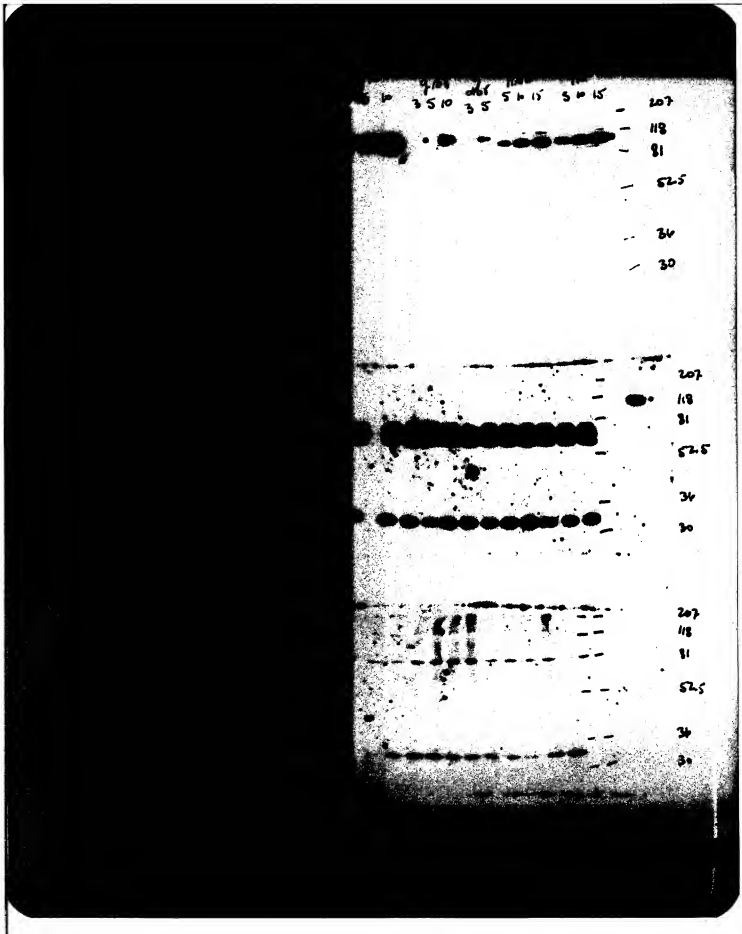
207
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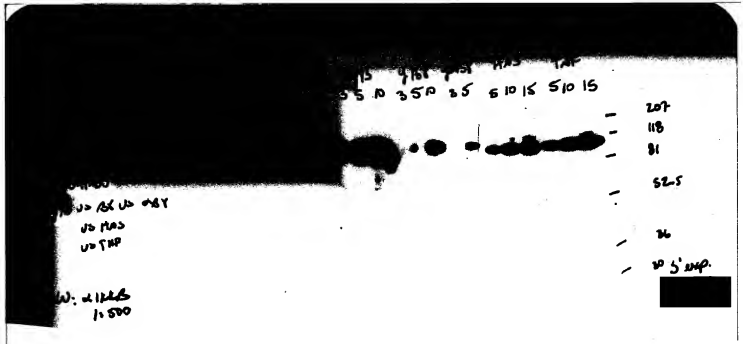
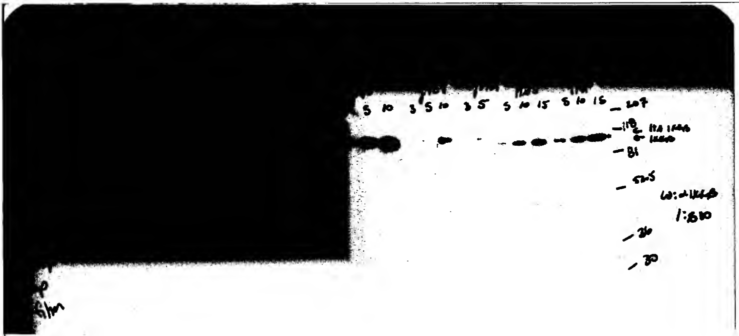

5 10 35 10 35 5 10 15 5 10 15
207
118
81
52.5
36
10 5' exp.



5 10 3 5 10 3 5 5 10 15 5 10 15
207
118
81
52.5
36
30

TOPS 36161
MADE IN U. S. A.

[redacted] Purpose: to compare IKK activity in
γβ vs γβγ vs γαβγ vs HNS vs TNF-Hela

repeat of 10-11 with attempt to use more similar amounts

HNS + TNF (Q20 → sup6 GF 10+11)
Put 300λ 1x IKinase buffer in bottom to prevent drying.
Top: 200λ sup6 GF 10 + 200λ sup6 GF 11

recover ~40λ + adjust vol. to 40λ (1x KA)

β-HA fraction 15 5λ + 45λ 1x
Dilute 1:10 in 1x kinase

Tube/lane
2 0.5λ 5λ + 16λ 1x
3 1λ 10λ + 11λ 1x
4 2λ 20λ + 1λ 1x

5 βγ - 7λ ~5λ (Hela TNF) + 14λ 1x
6 14λ ~10 + 7λ 1x
7 21λ ~15 +0

8 αβγ 7 +14λ 1x
9 14 +7λ 1x
10 21 +0

11 HNS 5 +16λ 1x
12 10 +11λ 1x
13 15 +6λ 1x

14 TNF 5 +16λ 1x
15 10 +11λ 1x
16 15 +6λ 1x
17 MW

1. Aliquot extract + buffer ~~30~~ 35λ
2. Add ~~35λ~~ Kinase cocktail Inc 30' 30°C
3. Add ~~10~~ 12λ 6x SDS PAGE
Heat 95°C 5'
4. Load 10% gel (40λ)

cocktail	16 samples +	4
10x kinase	48λ	12λ
20mM DTT	48λ	12λ
200μM ATP	48λ	12λ
GST-IkBα	32λ	8λ
^{32}P ATP	8λ	2λ
H_2O	296	74
	480	120

906-58

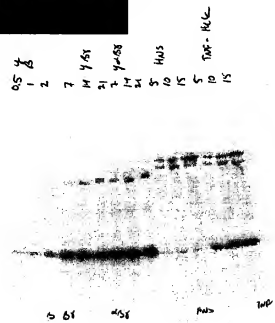
all loaded correctly 40λ each.

21
35
56

~~1M DTT~~ [illegible]
20 mM DTT
.02 1M + .98 H_2O

File/Range: D:\Users\1017bsm.gel / 0.000-45853 Counts / 0.814331
User Name: phospho
Image Name: D:\Users\1017bsm.gel
Image Comment: 2 experiments
1. 3 M urea GF column fractions (concentrated)
2. yeast b, bg, abg, HNS, TNF stim Hela

Present Date/Time: [redacted]
Scan Date/Time:
Prep. Date/Time:



scale 1-2500

12 yeast

File/Range: D:\Users\1017bsm.gel / 0.000-45853 Counts / 0.814331
User Name: phospho
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Image Comment: 2 experiments
1. 3 M urea GF column fractions (concentrated)
2. yeast b, bg, abg, HNS, TNF stim Hela

Present Date/Time: [redacted]
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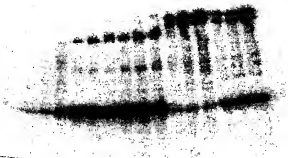

scale 1-250

[redacted]

kDa
30 kD.